Prevalence of Taenia solium cysticercosis in swine from a community-based study in 21 villages of the Eastern Cape Province, South Africa

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Abstract: The pork tapeworm, Taenia solium, causative organism of porcine cysticercosis and human neurocysticercosis is known to occur in areas of South Africa including Eastern Cape Province but, despite increasing reports of its occurrence throughout the subregion, the prevalence is yet to be clearly established. The parasite presents a potentially serious agricultural problem and public health risk in endemic areas. The human populations considered to be at highest risk of infection with this zoonotic helminth are people living in rural areas most of whom earn their livelihood wholly or partially through livestock rearing. Here we report on initial results of a community-based study of pigs owned by resource-poor, emerging pig producers from 21 villages in the Eastern Cape Province. Lingual examination (tongue palpation) in live pigs, two enzyme-linked immunosorbent assays (ELISAs), which detect parasite antigen (B158/B60 Ag-ELISA and HP10 Ag-ELISA) and an enzyme immunotransfer blot (EITB) assay, which detects antiparasite antibody, were used to verify endemicity and estimate apparent prevalence. In the absence of a gold standard true prevalence was obtained, using a Bayesian approach, with a model that uses both available data and prior information. Results indicate that the parasite is indeed present in the study villages and that true prevalence was 64.6%. The apparent prevalences as measured by each of the four tests were: 11.9% for lingual examination, 54.8% for B158/B60 Ag-ELISA, 40.6% for HP10 Ag-ELISA and 33.3% for EITB. This base-line knowledge of the prevalence of T. solium in pigs provides information essential to the design and monitoring of sustainable and appropriate interventions for cysticercosis prevention and control.

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Prevalence of *Taenia solium* cysticercosis in swine from a community-based study in 21 villages of the Eastern Cape Province, South Africa

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Abstract

The pork tapeworm, *Taenia solium*, causative organism of porcine cysticercosis and human neurocysticercosis is known to occur in areas of South Africa including Eastern Cape Province but, despite increasing reports of its occurrence throughout the subregion, the prevalence is yet to be clearly established. The parasite presents a potentially serious agricultural problem and public health risk in endemic areas. The human populations considered to be at highest risk of infection with this zoonotic helminth are people living in rural areas most of whom earn their livelihood wholly or partially through livestock rearing. Here we report on initial results of a community-based study of pigs owned by resource-poor, emerging pig producers from 21 villages in the Eastern Cape Province. Lingual examination (tongue palpation) in live pigs, two enzyme-linked immunosorbent assays (ELISAs), which detect parasite antigen (B158/B60 Ag-ELISA and HP10 Ag-ELISA) and an enzyme immunotransfer blot (EITB) assay, which detects antiparasite antibody, were used to verify endemicity and estimate apparent prevalence. In the absence of a gold standard true prevalence was obtained, using a Bayesian approach, with a model that uses both available data and prior information. Results indicate that the parasite is indeed present in the study villages and that true prevalence was 64.6%. The apparent prevalences as measured by each of the four tests were: 11.9% for lingual examination, 54.8% for B158/B60 Ag-ELISA, 40.6% for HP10 Ag-ELISA and 33.3% for

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EITB. This base-line knowledge of the prevalence of *T. solium* in pigs provides information essential to the design and monitoring of sustainable and appropriate interventions for cysticercosis prevention and control.

Keywords: Porcine cysticercosis; True and apparent prevalence; Resource-poor pig producers; Bayesian approach; Eastern Cape Province; South Africa; *Taenia solium*

1. Introduction

Many countries in Africa have reported high prevalences of *Taenia solium* cysticercosis/taeniasis whereas limited or no information is available on this infection in others (World Health Assembly, 2003). Within the subsaharan region, South Africa has the highest reported prevalence and is the country with the largest number of pigs of the eastern and southern African countries (Phiri et al., 2003; Krecek, 2005). Total pig numbers in South Africa have recently increased by 14% from 1,395,920 in 2000 to 1,590,837 in 2001 with similar trends reflected throughout each of the country's provinces (National Department of Agriculture Directorate, 2001). Approximately 25% of these pigs are free-ranging and are owned by emerging pig producers (i.e. pig owners striving to increase production above subsistence) in resource-poor areas of South Africa. Projections for pork consumption in the developing regions of the world for the period 1993–2020 are anticipated to double (39–81 million tonnes) compared with the developed regions from 38 to 41 million tonnes (ILRI, 2000).

A recent review of the current status of human neurocysticercosis (NCC) in eastern and southern Africa included prevalences of infected pigs from slaughterhouses of 0.5–25.7% (Mafojane et al., 2003). This paper also highlighted the need for community-based studies of both health and agriculture aspects of this zoonotic disease. In response to this, an epidemiologic study funded by the United States Agency for International Development (USAID) in South Africa addressed porcine *T. solium* infections in emerging farming areas of the Eastern Cape Province where the highest levels of NCC have been reported (Mafojane et al., 2003; Krecek et al., 2004; Phiri et al., 2003). In humans, hospital surveys utilizing serological and radiological diagnostic techniques reported that 28–50% of African children with epilepsy were positive for cysticercosis (Phiri et al., 2003). The first objective of this, community-based, study was to verify endemicity and estimate the prevalence of *T. solium* cysticercosis in pigs. Due to cost constraints and local socio-economic and infrastructural conditions, however, important baseline prevalence data on *T. solium* cysticercosis in pigs could not be obtained using the 'gold standard' technique of total carcass dissection. However, lingual examination (tongue palpation) in live pigs, two enzyme-linked immunosorbent assays (ELISAs), which detect parasite antigen (B158/B60 Ag-ELISA and HP10 Ag-ELISA) and an enzyme immunotransfer blot (EITB) assay, which detects antiparasite antibody, were used, and the results of these tests together with prior information on the test performances were processed in a Bayesian analysis to estimate the true prevalence. The second objective was to gain a better understanding of pig husbandry practices, pork consumption, sanitation and people's knowledge of this parasite in communities where NCC and cysticercosis occur through questionnaire survey techniques. The final objective was to identify appropriate recommendations for prevention and management of this parasite. The current paper reports on the results of the first study.

2. Materials and methods

2.1. Epidemiological design and sampling procedure

From February to June 2003, 21 villages in the Alfred Nzo and Oliver R. Tambo Districts of the Eastern Cape Province of South Africa were visited (Fig. 1), blood samples from 261 pigs were drawn and 122 questionnaires and interviews with resource-poor emerging pig producers completed. Matatiele, which is included in Fig. 1 is not a study village. It is included because this is where the abattoir was located. Provincial veterinary activities focus around dip tanks and associated villages in the six veterinary districts of the Eastern Cape Province. Villages with emerging pig producers, which were accessible by road were identified in both districts. In Oliver R. Tambo District there were 124 tanks/villages and approximately 17,964 pigs. In Alfred Nzo District there were 16,414 pigs. Twelve and nine villages were selected for Oliver R. Tambo and Alfred Nzo Districts, respectively, based on the criteria above. In these remote rural areas, distances between the homes of individual pig owners were often large (0.5–1.0 km). Walking was the only access. Visiting 15 owners, conducting interviews

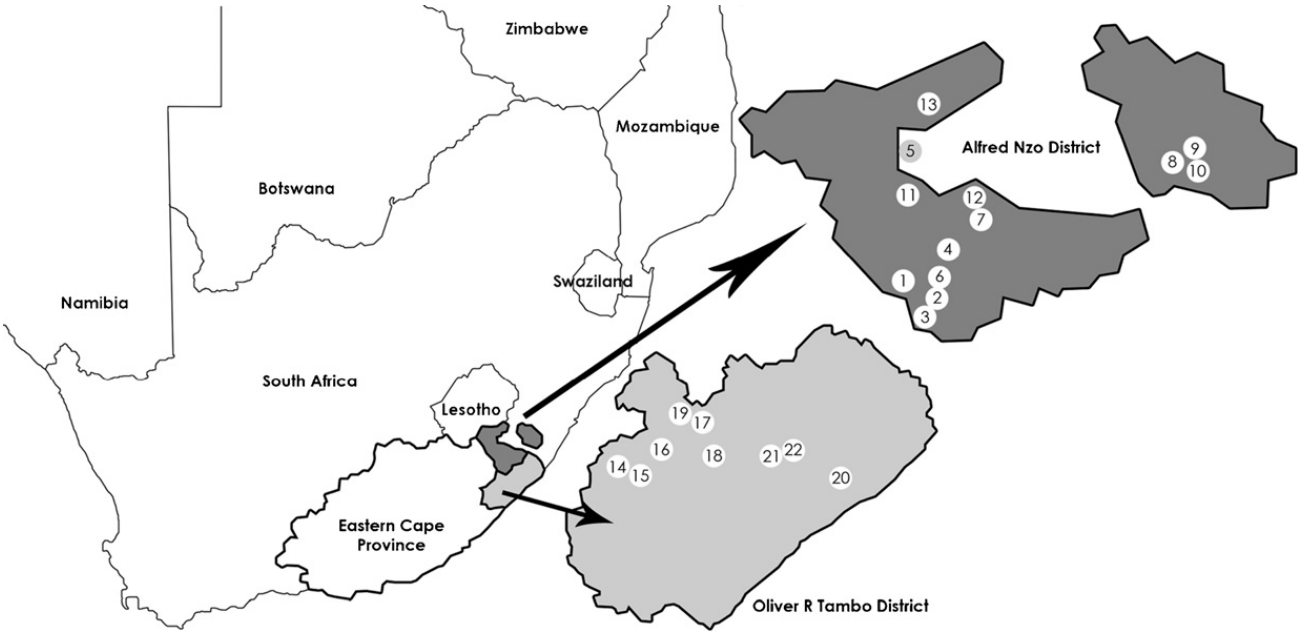

Fig. 1. The study took place in 21 villages in the Alfred Nzo and Oliver R. Tambo Districts of the Eastern Cape Province, South Africa. Thirteen villages of the Alfred Nzo District were: (1) Mt Frere (Mvuzi), (2) Mt Frere (Tshungwana), (3) Mt Frere (Mhlanganisweni), (4) Mt Frere (Cabazi), (5) Matatiele, (6) Mt Frere (Mtshazi), (7) Mt Frere (Colana), (8) Umzimkulu (Ntshongo Kromdraai 2), (9) Umzimkulu (Mgwala), (10) Umzimkulu (Mvolozana), (11) Maluti (Sidakeni), (12) Maluti (Gwadana) and (13) Maluti (Afsondering). Matatiele is included because this is where the abattoir is located and was not a study village. Nine villages of the Oliver R. Tambo District included: (14) Tsolo (Mbinja), (15) Tsolo (Xabane), (16) Tsolo (Mhlabati), (17) Qumbu (Ngxakolo), (18) Qumbu (Kwam), (19) Qumbu (Ngcolokini), (20) Lusikisiki (Nkunzimbini), (21) Lusikisiki (Hlababomvu), and (22) Lusikisiki (Mfinizweni).

and sampling pigs was the maximum the team could access in one day. Collection days were identified and based on the estimate of 15 pig owners per day. Representative owners were randomly selected to take part. Visits were scheduled for those who agreed to participate. Most owners owned 1–2 pigs. All pigs of each owner were sampled. Therefore within the constraints of the study (i.e. accessibility by road, distances to travel, difficulty for owners to be available some days when the collection team visited, etc.) the owners and pigs were representative for the population of free-ranging pigs of the study area.

Data was captured and summarized using the statistical program GenStat (2003) and Microsoft (2002).

2.2. Lingual examination and pig sampling

The free ranging pigs were largely South African hut pigs. This is an indigenous local small and usually black pig commonly raised in this area. The tongues of the pigs were first examined for the presence of *T. solium* cysticerci (lingual examination). Each pig was laid on its back and maintained in a straight line during handling. The animal was restrained manually and mutton cloth strips used to hold its mouth open (modified from Mathias Boa, personal communication, 2003). This diagnostic method used in the current study follows: the tongue was grasped gently but firmly with Guy's 19.5 cm (7.5″) tongue holding forceps and extended from the mouth cavity, while a torch (Search Guard, 1,000,000 cd Quartz Halogen Lantern) was used for illumination. This lantern helped to illuminate the pig's mouth well, even when working in bright sunlight, and was easily recharged in the field by use of an integral car cigarette lighter plug.

Blood samples were collected from the cranial vena cava of the pigs using standard syringes and heparinized vacutainer tubes (18 gauge needles). The blood samples were then processed and the serum aliquoted and stored in labeled cryogenic vials at -20 °C until use. Sex, age and body condition scoring values of each pig were recorded (D.P. Visser, ARC-Animal Improvement Institute, South Africa, 2002).

2.3. Serodiagnostic testing

2.3.1. Enzyme-linked immunosorbent assays (ELISAs)

The two monoclonal antibody-based parasite antigen tests, which were employed included the B158/B60 Ag-ELISA (Dorny et al., 2000) and the HP10 Ag-ELISA (Harrison et al., 1989; Harrison and Sewell, 1991).

In the B158/B60 Ag-ELISA the calculation of the cut off is based on the results of eight negative controls: the optical density of each serum sample is compared with the mean OD of a series of eight samples from non-infected individuals at a probability level of $P < 0.001$ to determine the result in the test (Sokal and Rohlf, 1981). The calculation is based on the modified student *T*-test.

The presence of HP10 *T. saginata* metacestode antigen in the serum of the study pigs was detected using the specific McAb HP10-based ELISA capture assay (HP10 Ag-ELISA) (Harrison et al., 1989) with slight modification as described by Sciutto et al. (1998) and Harrison et al. (2005), and employing Streptavidin Peroxidase (Pierce Ltd., 0.1 μg/ml), followed by tetramethylbenzidine liquid substrate (Sigma Ltd.) as the indicator system. ELISA plates were routinely set-up to include six duplicate positive and six duplicate negative controls and four diluent controls, while each test sample was run in duplicate. The mean sample ODs minus the mean diluent ODs were corrected for any day-to-day variation using a correction factor basically as described by Harrison et al. (2005). ELISA results were rejected if the correction factor for any particular plate varied more than 10% from the reference day. The optical density (OD) of each serum sample was compared with the negative serum control samples at a probability level of $P = 0.001$. This was used to determine the negative cut-off point and hence distinguish between the positive and negative results of the Ag-ELISA. A cut-off point was determined for each plate of both the HP10 Ag-ELISA and the B158/B60 Ag-ELISA.

2.3.2. Enzyme-linked immunoelectrotransfer blot (EITB)

An enzyme-linked-immunoelectrotransfer blot assay (EITB) based on affinity purified glycoproteins from *T. solium* cysticerci (Tsang et al., 1989; Tsang et al., 1991) was used for detection of antibodies in serum. Samples were tested twice on different days to determine the reproducibility of the testing in the laboratory. A sample was considered positive if at least one of the seven antigens (molecular weight 50, 42–39, 24, 21, 18, 14 and 13 kDa) was recognized.

2.3.3. Interpretation of biotechnological diagnostic tests

None of the conventional available tests is perfect (sensitivity and specificity of hundred percent). As mentioned previously complete dissection and recovery of cysticerci is the 'gold standard' for diagnosis of *T. solium*, but was not carried out in the current study due to cost constraints and local socio-economic and infrastructural conditions. Moreover, in the absence of a 'gold standard' and considering a possible conditional dependence between the diagnostic tests, more parameters must be estimated than the data permit, making estimation of the true prevalence of the disease and of the test characteristics impossible. Therefore, the results of the four tests were integrated together with expert opinions (prior information) in a Bayesian model, based on a multinomial distribution (Lesaffre et al., 2007; Dorny et al., 2004). The use of the prior information allowed the reduction of the number of parameters to be estimated (31) to the number of estimable parameters (15) (Berkvens et al., 2006). The prior information on test characteristics was obtained from helminthologists at the Institute of Tropical Medicine of Antwerp and at the University of Edinburgh (Appendix A). The model was constrained as follows: the sensitivity and the specificity of the tongue palpation were constrained uniformly to interval [0,0.3] and [0.7,1], respectively; the sensitivity of the B158/B60 Ag-ELISA was constrained by a uniform prior on th[4] to interval [0.9,1]; the specificity of the B158/B60 Ag-ELISA was constrained by a uniform prior on th[6] to interval [0.9,1]; the sensitivity of the HP10 Ag-ELISA was constrained by a uniform prior on th[8] to interval [0.4,1]; the specificity of the HP10 Ag-ELISA was constrained by a uniform prior on th[12] to interval [0.4,1]; the sensitivity of the EITB was constrained by a uniform prior on th[16] to interval [0.9,1]; the specificity of the EITB was constrained by a uniform prior on th[24] to interval [0.9,1]. Th[4], th[6], th[8], th[12], th[16] and th[24] are conditional probabilities described by Berkvens et al. (2006). The criterion used for the presence of the parasite was that either of the Ag-ELISA tests was positive. There was one village, Tshungwana, in which the B158/B60 Ag-ELISA test was positive and negative with the HP10 Ag-ELISA. The presence or absence of metacestodes of *T. hydatigena* in pigs in the area was decisive to allow the use of constraints on the specificity of the Antigen ELISA tests, since cross-reactions were described with the parasite in these tests. Horak (1980) reported few cases of *T. hydatigena* in pigs examined at an abattoir in Gauteng province, South Africa. In addition, the prevalence of this parasite in Zambian village pigs was reported to be low and consequently did not seem to interfere much with serological test results for diagnosis of *T. solium* in southern Africa (Dorny et al., 2004).

The analysis was performed in the WinBUGS program using three chains of 20,000 iterations and a burn-in of 5000 (Lunn et al., 2000). The convergence between the three chains was checked and the model was validated using the criteria proposed by Berkvens et al. (2006). The correspondence between the p_D (number of parameters estimated by the model) and DIC (Deviance Information Criterion) values calculated in the posterior mean of the multinomial probabilities and in the posterior mean of the parameters of the model (parent nodes) was checked. Finally, the trend of the *Bayesp* (Bayesian *p*-value) towards 0 when narrowing the constraints on the estimates was verified.

3. Results

The results of the dip tanks and villages visited, the numbers of pigs examined, the lingual examinations of the pigs and the three serological tests and the percentages positive are presented in Table 1. The results of the estimates of true prevalence, sensitivities' and specificities' based on Bayesian analysis of the four diagnostic tests used in pigs in 21 villages in the Eastern Cape Province, South Africa are provided in Table 2. The validation criteria for this analysis are in Table 3. Table 4 includes the results of the diagnostic tests performed and provides a comparison of the groups of tests.

The pigs ranged in age 2–60 months (mean = 12.55 months), weights 1–150 kg (mean = 23.82 kg) and body condition score 1–5 (mean = 2.01).

As judged by the lingual examination, the overall mean prevalence for 261 pigs was 11.9% and the parasite was present in 13/21 of the villages. The percentage of 261 pigs identified as positive by Ag-ELISA was higher at 54.8% and 40.6%, respectively as estimated by the B158/B60 Ag-ELISA and HP10 Ag-ELISA. Indications were that the parasite was present in all but one of the villages. Finally, overall, 33.3% of 261 pigs were antibody-positive as judged by the EITB assay, with indications that pigs in all but one of the villages had been exposed to infection.

The Bayesian analysis estimated the true prevalence of porcine cysticercosis at 64.6%.

4. Discussion

Due to cost constraints and local socio-economic and infrastructural conditions, baseline prevalence data on *T. solium* cysticercosis in pigs was not obtained using the 'gold standard' technique of total carcass dissection. This method is used to indicate levels of infection for porcine cysticercosis with examination of the total number of cysts in the pig carcass. This would require purchasing animals from owners, dissecting all muscles and recovering all cysticerci. The pig owners in this study were reluctant to sell their animals because of unavailability of replacement animals. In addition, resources in the current project were limited. Therefore, a Bayesian approach based on results of multiple diagnostic tests was used as an alternative to estimate the true prevalence of the disease in a population of 261 pigs sampled in the Eastern Cape Province of South Africa. The results of the four tests were computed together with expert opinions in a Bayesian model and gave an estimation of the true prevalence of 64.6%, which is close to prevalence estimated by Dorny et al. (2004) using the same approach in a Zambian village pig population (64.2%). These findings show a very high endemicity of *T. solium* in these two regions of southern Africa, where all risk factors maintaining the life cycle of the parasite are present: free roaming of pigs, absence or irregular use of latrines and no official inspection of the pig carcasses. Both estimates are remarkably higher than those reported in South Africa by Phiri et al. (2003), which ranged between 0% and 25%. Those estimates, however, were based on the results of a single test, namely the routine carcass inspection, known as a rather unsensitive diagnostic method (Phiri et al., 2006).

The Eastern Cape Province is challenged in terms of infrastructure, unemployment and the economy. When compared with national statistics, unemployment is higher (55% vs. 42%), homes with piped water lower (62% vs. 84%) and fewer homes with toilets (14% vs. 31%). Such statistics reveal the socioeconomic context in which porcine cysticercosis currently occurs and how this environment may impact on the success of recommendations and interventions (Statistics South Africa, 2001). The presence of *T. solium* limits pork production and is a serious threat to public health in this Province (Krecek, 2005).

The lingual examination method is used by resource-poor farmers and reported to be relatively sensitive and highly specific for detecting *T. solium* infected animals in Peru (Gonzalez et al., 1990). The method in the current study was similar but differed in placing the pig on its back and not its side, using mutton cloth only and no rod, using visual inspection and no palpation. Bright illumination and a forceps for grasping the tongue were added to this method in the current study.

This study also provided estimates of the performances of the four diagnostic tools used in the survey. The estimate of the sensitivity of the tongue

Table 1
The percentage (%) of pigs diagnosed as positive for porcine cysticercosis by four diagnostic tests (lingual examination, B158/B60 Ag-ELISA, HP 10 Ag-ELISA and the EITB)

Dip tank (village)	% of pigs diagnosed as positive				
	Number of pigs examined	Lingual examination	B158/B60 Ag-ELISA	HP10 Ag-ELISA	EITB
Mt Frere					
Mvuzi	5	20.0	60.0	60.0	60.0
Tshungwana	4	25.0	25.0	0	50.0
Mhlanganisweni	8	25.0	50.0	37.5	50.0
Cabazi	11	0	81.8	63.6	54.6
Mtshazi	15	20.0	33.3	26.7	40.0
Colana	14	28.6	57.1	35.7	50.0
Umzimkulu					
Ntshongo Kromdraai 2	19	26.3	47.4	36.8	31.6
Magwala	13	15.4	76.9	61.5	15.4
Mvolozana	19	0	36.8	31.6	21.1
Maluti					
Sidakeni	8	0	50.0	50.0	50.0
Gwandana	17	5.9	58.8	41.2	5.9
Afsondering	11	0	36.4	9.1	0
Tsolo					
Mbinja	13	38.5	84.6	53.9	23.1
Xabane	17	0	64.7	41.2	11.8
Mhlabati	16	0	68.8	56.3	25.0
Ngxakolo	14	7.1	78.6	50.0	35.7
Qumbu					
Kwam	13	0	76.9	53.9	61.5
Ngcolokini	13	15.4	53.9	46.2	53.9
Nkunzimbini	11	0	36.4	36.4	63.6
Lusikisiki					
Hlababomvu	7	14.3	28.6	28.6	14.3
Mfinizweni	13	23.1	15.4	15.4	38.5
Overall	261	11.9	54.8	40.6	33.3

The results are based on 261 pigs from 21 villages in Eastern Cape Province, South Africa.

Table 2
The estimates of true prevalence (true prev), sensitivities' (se) and specificities' (sp) based on Bayesian analysis of the four diagnostic tests used in pigs from 21 villages in the Eastern Cape Province, South Africa

True prev	0.646 (0.518–0.827)[a]
Lingual examination	
Se	0.082 (0.018–0.149)
Sp	0.802 (0.708–0.904)
B158/B60 Ag-ELISA	
Se	0.763 (0.609–0.886)
Sp	0.841 (0.744–0.933)
HP10 Ag-ELISA	
Se	0.548 (0.423–0.664)
Sp	0.833 (0.715–0.925)
EITB	
Se	0.453 (0.355–0.550)
Sp	0.853 (0.765–0.931)

[a] 95% credibility intervals.

examination was very low (less than 10%) demonstrating the weakness of this inspection method to detect cysticercosis in pigs and explaining the severe underestimation of the true prevalence (about six times higher than the apparent prevalence estimated by tongue palpation in this study). On the other hand, the estimate of the specificity of the method is high but less than 100%, as was described in other reports (Dorny et al., 2004; Phiri et al., 2006). These estimates underline the difficulty to perform such an inspection in the field and to standardize its protocol, depending on the experience of the executor and on the intensity of infection in the population tested (Sciutto et al., 1998).

The performances of the two available ELISAs (B158/B-60 and HP10) detecting circulating antigens of the metacestode of *T. solium* were compared here for the first time. The specificities' estimates of both B158/B60 and HP10 ELISA tests were almost similar,

Table 3
Validation criteria of the Bayesian analysis selected to estimate the prevalence and diagnostic test characteristics of porcine cysticercosis in the Eastern Cape Province, South Africa

Bayesp	0.4561
p_D using posterior means of the multinomial probabilities	11.846
p_D using parent nodes	11.514
DIC using posterior means of the multinomial probabilities	78.122
DIC using parent nodes	77.914

p_D is the number of parameters estimated by the model, Deviance information criterion (DIC) values and parent nodes are the parameters of the model.

Table 4
Results obtained with four different tests to detect porcine cysticercosis in African hut pigs in the Eastern Cape Province, South Africa

Tongue palpation	B158/B60 Ag-ELISA	HP10 Ag-ELISA	EITB	Number of pigs
0	0	0	0	80
0	0	0	1	25
0	0	1	0	3
0	0	1	1	2
0	1	0	0	32
0	1	0	1	6
0	1	1	0	43
0	1	1	1	39
1	0	0	0	5
1	0	0	1	1
1	0	1	0	2
1	0	1	1	0
1	1	0	0	3
1	1	0	1	3
1	1	1	0	6
1	1	1	1	11

0 = negative test result; 1 = positive test result.

with 84.1% and 83.3%, respectively. However, the sensitivities' estimates vary with 76.3% for B158/B60 and 54.8% for HP10. These two tests use different monoclonal antibodies to capture circulating antigens. These may not capture the same antigen or react with the same epitope, explaining such sensitivity estimates differences.

The sensitivity estimate for the EITB was low. This is mainly due to the fact that 43 animals were positive for both Ag-ELISAs and negative for both tongue palpation and EITB. This could be due to a higher sensitivity of Ag-ELISA compared to antibody detection (Dorny et al., 2004).

Finally, the ultimate goal of the field work on porcine cysticercosis was to identify promising interventions and formulate appropriate recommendations for prevention and management of *T. solium* in this area. The results presented here have clearly confirmed the widespread endemicity of the parasite in the study area and give base-line estimations of prevalence. This information combined with associated questionnaire studies will greatly contribute to this goal.

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Appendix A

Model for WinBUGS including the results of the four diagnostic tests and the prior information

```
model
{
r[1:16] ~ dmulti( p[1:16], n )
pr[1] <- th[1]*(1-th[2])*(1-th[5])*(1-th[11])*(1-th[23])+(1-th[1])*th[3]*th[6]*th[12]*th[24]
pr[2] <- th[1]*(1-th[2])*(1-th[5])*(1-th[11])*th[23]+(1-th[1])*th[3]*th[6]*th[12]*(1-th[24])
pr[3] <- th[1]*(1-th[2])*(1-th[5])*th[11]*(1-th[22])+(1-th[1])*th[3]*th[6]*(1-th[12])*th[25]
pr[4] <- th[1]*(1-th[2])*(1-th[5])*th[11]*th[22]+(1-th[1])*th[3]*th[6]*(1-th[12])*(1-th[25])
pr[5] <- th[1]*(1-th[2])*th[5]*(1-th[10])*(1-th[21])+(1-th[1])*th[3]*(1-th[6])*th[13]*th[26]
pr[6] <- th[1]*(1-th[2])*th[5]*(1-th[10])*th[21]+(1-th[1])*th[3]*(1-th[6])*th[13]*(1-th[26])
pr[7] <- th[1]*(1-th[2])*th[5]*th[10]*(1-th[20])+(1-th[1])*th[3]*(1-th[6])*(1-th[13])*th[27]
pr[8] <- th[1]*(1-th[2])*th[5]*th[10]*th[20]+(1-th[1])*th[3]*(1-th[6])*(1-th[13])*(1-th[27])
pr[9] <- th[1]*th[2]*(1-th[4])*(1-th[9])*(1-th[19])+(1-th[1])*(1-th[3])*th[7]*th[14]*th[28]
pr[10]<- th[1]*th[2]*(1-th[4])*(1-th[9])*th[19]+(1-th[1])*(1-th[3])*th[7]*th[14]*(1-th[28])
pr[11]<- th[1]*th[2]*(1-th[4])*th[9]*(1-th[18])+(1-th[1])*(1-th[3])*th[7]*(1-th[14])*th[29]
pr[12]<- th[1]*th[2]*(1-th[4])*th[9]*th[18]+(1-th[1])*(1-th[3])*th[7]*(1-th[14])*(1-th[29])
pr[13]<- th[1]*th[2]*th[4]*(1-th[8])*(1-th[17])+(1-th[1])*(1-th[3])*(1-th[7])*th[15]*th[30]
pr[14]<- th[1]*th[2]*th[4]*(1-th[8])*th[17]+(1-th[1])*(1-th[3])*(1-th[7])*th[15]*(1-th[30])
pr[15]<- th[1]*th[2]*th[4]*th[8]*(1-th[16])+(1-th[1])*(1-th[3])*(1-th[7])*(1-th[15])*th[31]
pr[16]<- th[1]*th[2]*th[4]*th[8]*th[16]+(1-th[1])*(1-th[3])*(1-th[7])*(1-th[15])*(1-th[31])
for (i in 1:16)
{
        d[i] <- r[i]*log(max(r[i],1)/(p[i]*n))
}
G0 <- 2 * sum(d[])
r2[1:16] ~ dmulti(p[1:16], n)
for (i in 1:16)
{
        d2[i] <- r2[i]*log(max(r2[i],1)/(p[i]*n))
}
Gt <- 2 * sum(d2[])
bayesp <- step(G0 - Gt)
th[1]   ~ dbeta(1,1)
th[2]   ~ dbeta(1,1)I(0,0.3)
th[3]   ~ dbeta(1,1)I(0.7,1)
th[4]   ~ dbeta(1,1)I(0.9,1)
th[5]   ~ dbeta(1,1)
th[6]   ~ dbeta(1,1)I(0.9,1)
th[7]   ~ dbeta(1,1)
th[8]   ~ dbeta(1,1)I(0.4,1)
th[9]   ~ dbeta(1,1)
th[10]  ~ dbeta(1,1)
th[11]  ~ dbeta(1,1)
th[12]  ~ dbeta(1,1)I(0.4,1)
```

```
th[13]  ~ dbeta(1,1)
th[14]  ~ dbeta(1,1)
th[15]  ~ dbeta(1,1)
th[16]  ~ dbeta(1,1)I(0.9,1)
th[17]  ~ dbeta(1,1)
th[18]  ~ dbeta(1,1)
th[19]  ~ dbeta(1,1)
th[20]  ~ dbeta(1,1)
th[21]  ~ dbeta(1,1)
th[22]  ~ dbeta(1,1)
th[23]  ~ dbeta(1,1)
th[24]  ~ dbeta(1,1)I(0.9,1)
th[25]  ~ dbeta(1,1)
th[26]  ~ dbeta(1,1)
th[27]  ~ dbeta(1,1)
th[28]  ~ dbeta(1,1)
th[29]  ~ dbeta(1,1)
th[30]  ~ dbeta(1,1)
th[31]  ~ dbeta(1,1)

se[1] <- th[2]
se[2] <- th[4]*th[2]+th[5]*(1-th[2])
se[3] <- th[8]*th[4]*th[2]+th[9]*(1-th[4])*th[2]+th[10]*th[5]*(1-th[2])+th[11]*(1-th[5])*(1-th[2])
se[4] <- th[2]*(th[4]*(th[8]*th[16]+(1-th[8])*th[17])+(1-th[4])*(th[9]*th[18]+(1-th[9])*th[19]))+(1-
th[2])*(th[5]*(th[10]*th[20]+(1-th[10])*th[21])+(1-th[5])*(th[11]*th[22]+(1-th[11])*th[23]))

sp[1] <- th[3]
sp[2] <- th[6]*th[3]+th[7]*(1-th[3])
sp[3] <- th[12]*th[6]*th[3]+th[13]*(1-th[6])*th[3]+th[14]*th[7]*(1-th[3])+th[15]*(1-th[7])*(1-th[3])
sp[4] <- th[3]*(th[6]*(th[12]*th[24]+(1-th[12])*th[25])+(1-th[6])*(th[13]*th[26]+(1-
th[13])*th[27]))+(1-th[3])*(th[7]*(th[14]*th[28]+(1-th[14])*th[29])+(1-th[7])*(th[15]*th[30]+(1-
th[15])*th[31]))

}

list(r=c(80,25,3,2,32,6,43,39,5,1,2,0,3,3,6,11), n=261)
```

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